Prevalence of Metallo-β-lactamases Producing *Escherichia coli* Isolated from North of Palestine

**ABSTRACT**

**Objective:** This study aimed to determine the prevalence of Metallo-β-lactamases (MBLs) among clinical *E. coli* isolated from North of Palestine.

**Methods:** A total of 79 of *E. coli* isolates were recovered from hospitals in North of Palestine during February-July 2015. A multiplex PCR was used to determine the prevalence of MBLs among these isolates.

**Results:** Results showed that the prevalence of MBLs was 87.4%. The *spm* gene was the most prevalent (86.1%) among these isolates. According to the geographical distribution, results showed that the prevalence of MBLs among *E. coli* isolates recovered from Thabet Hospital-Tulkarm was 75.9%. The *Spm* gene was the most common (72.4%) among these isolates, while the *Imp* gene was detected in 41.4% of isolates. For isolates recovered from Jenin Hospitals, the prevalence was 94% and *spm* gene was detected in all MBL producers, 2 other genes were detected, *Imp* and *Sim*, and the prevalence was 12% and 2%, respectively. A total of 26.1% of MBL *E. coli* producers carried 2 genes.

**Conclusions:** Our results showed high occurrence of MBLs among *E. coli* isolates in Palestine. Based on these results we recommend the continuous monitoring and surveillance of the prevalence, proper control and prevention practices and effective antibiotic use will limit the further spread of MBLs producing isolates within hospitals in this country. Prevalence of MBL producing microorganisms has not been investigated previously.

**KEY WORDS:** Metallo-β-lactamases

*E. coli*

MBLs

Palestine

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**Introduction**

Metallo-beta-lactamase (MBL) producing Gram-negative bacteria are being reported with increasing frequency from several countries and have emerged as a most widespread and clinically significant carbapenem resistance mechanism [1]. Metallo-β-lactamase producing pathogens can hydrolyze all types of β-lactams including penicillins, cephalosporins, carbapenems, cephemycins, except monobactams as aztreonam [2]. In addition, their catalytic activities are not affected by available β-lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam [3]. These enzymes belong to Ambler class B β-lactamases based on their amino acid sequence homology and to group 3 according to the Bush classification based on their substrate and inhibitor profiles [2]. Class B β-lactamase are carbapenemases type which depend on zincons to hydrolyze the β-lactams and due to this, the presence of divalent cation-chelating agents such as EDTA, MBL catalysis is inhibited [4,5]. Metallo-β-lactamases enzymes are either chromosomally encoded (resident MBLs) mainly in environmental bacteria or opportunistic pathogens or clinically relevant metallo-β-lactamases are carried on highly mobile genetic elements allowing easy dissemination (acquired or plasmid mediated MBLs) [6,7]. The MBLs which are produced by environmental bacteria, could be precursors for the efficient MBLs present in clinical pathogens that are exposed to increasing doses of antibiotics [8].

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**References**

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Enterobacteriaceae such as Escherichia coli and Klebsiella pneumoniae [11,12]. In 2011, outbreaks of NDM-1-producing E. coli and Klebsiella pneumoniae isolates have been obtained from patients hospitalised in four healthcare facilities in northern Italy [13]. Recently, a study in Nepal showed that the incidence of MBL among E. coli recovered from different clinical isolates was 18.98% [12]. Several studies in India showed the prevalence of MBL producing E. coli ranged from 1.7%-15% [14-18]. In addition, in India it was shown that 50%-81.8% of E. coli resistant/intermediate to carbapenems were MBL producers [19-21]. In Pakistan, it was reported that 71% carbapenem-resistant E. coli were MBL producers [22]. In a PCR-Based Nosocomial Surveillance Study in Puerto Rico[23], it was found 5.1% of E. coli isolates were MBLs producers. In Nigeria, it was found that the prevalence of MBLs among E. coli ranged from 12.5%-41.2% [24,25]. In Iraq, 45.2% of E. coli isolated from pregnant and non-pregnant women with genital tract infection were MBL producers [26].

NDM MBL producing E. coli was detected in Poland [27]. In Algeria, the first three autochthonous cases of infections caused by NDM-5 MBL-producing Escherichia coli strains were recovered from urine and blood specimens, these isolates were coexpressed blaCTX-M-15 with the blaTEM-1 and blaaadA2 genes [28]. In China, the first description of IncX3 plasmids carrying NDM-1 β-lactamases in E. coli was reported [29]. In Japan, 90.7% of MBL producing E. coli carried IMP-6 gene and 9.3% carried IMP-1 gene, all IMP-6-positive isolates were carried CTX-M. 47% of IMP-6-positive isolates were positive for TEM [30]. VIM-1-β-lactamases-producing E. coli has been detected in a university hospital in Greece [31]. The first report of IMP-1-producing E. coli in Turkey was described by Aktas [32]. In other study in Turkey, 0.0% of multidrug resistant E. coli isolated from a tertiary care training and research hospital were producing MBLs [33]. The first report about detection of the blaNDM-1 MBL gene in Australia was described previously [34].

This study was conducted to address part of deficient information in molecular antibiotic resistance characterization and their transmissible potential in Palestine. Therefore, this study aimed to determine the prevalence of MBLs among clinical E. coli isolated from North of Palest-
for 5 min. The PCR products were detected by electrophoresis through 1.5% agarose gels to determine the size of amplified fragment after ethidium bromide staining.

**Table 1.** Target genes for PCR amplification, amplicon size, primer sequences and annealing temperature.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer sequence 5’→3’</th>
<th>Annealing temperature</th>
<th>Amplicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Imp</em></td>
<td>Imp-F GGA ATA GAG TGG CTT AAY TCT C Imp-R CCA AAC YAC TAS GTT ATC T</td>
<td>52°C</td>
<td>188bp</td>
</tr>
<tr>
<td><em>Vim</em></td>
<td>Vim-F GAT GGT GTT TGG CAT CA Vim-R S CCA ATG CCG AGC ACC AG-3</td>
<td>52°C</td>
<td>390bp</td>
</tr>
<tr>
<td><em>Gim</em></td>
<td>Gim-F TGC ACA CAC CTT GGT CGT AA Gim-R AAC TCT CAA CTG TGC CAT GC</td>
<td>52°C</td>
<td>477bp</td>
</tr>
<tr>
<td><em>Spm</em></td>
<td>Spm-F AAA ATC TGG GTA CGC AAA CG Spm-R ACA TTA TCC GCT GGA ACA GG</td>
<td>52°C</td>
<td>271bp</td>
</tr>
<tr>
<td><em>Sim</em></td>
<td>Sim-F TAC AAG GGA TTC GCC ATC G Sim-R TAA TGG CCT GTT CCC ATG TG</td>
<td>52°C</td>
<td>570bp</td>
</tr>
</tbody>
</table>

ERIC (Enterobacterial repetitive intergenic consensus) PCR was performed using Primer ERIC1: 5’-ATG TAA GCT CCT GGG GAT TCA C-3’ and Primer ERIC2: 5’-AAG TAA GTG ACT GGG GTG AGC G-3’. Each PCR reaction mix was performed in a final volume of 25 μL containing 12.5 μL of PCR premix with MgCl₂ (Ready-MixTM Taq PCR Reaction Mix with MgCl₂, Sigma), 1 μM of each primer, 3 μL DNA template. In addition, the master mix was modified by increasing the concentration of dNTPs to 0.4 mM, 3 mM MgCl₂ and 1.5 U of Taq DNA polymerase. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 2 min at 94°C was followed by 30 cycles of initial denaturation 94°C for 50 s, 50°C for 40 s and 72°C for 1 min, with a final extension step at 72°C for 5 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel stained with ethidium bromide. The gel images were scored using a binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively. A binary matrix was analyzed by the unweighted pair group method for arithmetic averages (UPGMA), using SPSS statistics software version 20 (IBM).

**Results**

The antimicrobial susceptibility pattern revealed that the these clinical *E. coli* strains were less resistant (24.1%) to Kanamycine and Ceftriaxone, while showed high resistant to Trimethoprim/Sulfamethoxazole (67.1%) and Tetracycline (60.8%). Data are presented in Table 2.

**Table 2.** Antibiotic resistance of 79 *E. coli* isolates recovered from different clinical samples-Palestine.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant strains %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>38.0%</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>67.1%</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>24.1%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>60.8%</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>36.7%</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>29.1%</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>24.1%</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>41.8%</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>41.8%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>38%</td>
</tr>
<tr>
<td>Imipenem</td>
<td>44.3%</td>
</tr>
</tbody>
</table>

Our results showed that the prevalence of MBLs among *E. coli* isolated from North of Palestine was 87.4%. The *spm* gene was the most prevalent (86.1) among these isolates. According to the places, results showed that the prevalence of MBLs genes in *E. coli* isolates recovered from Thabet Hospital Tulkarm using multiplex PCR technique was 75.9%. The *Spn* gene was the most common (72.4%) among these isolates, while the *Imp* gene was detected in 41.4% of isolates. Isolates recovered from Jenin Hospitals, the prevalence was 94% and *spn* gene was detected in all MBL producers. Other 2 genes were detected, *Imp* and *Sim*, and the prevalence was 12% and 2%, respectively. A total of 26.1% of MPL producers carried 2 genes. Data are presented in Table 3 and Figure 1.

**Table 3.** Distribution of MBL genes among *E. coli* isolated from Jenin and Tulkarm hospitals.

<table>
<thead>
<tr>
<th>Location (N)</th>
<th><em>spn</em> (40 (80))</th>
<th><em>spn+Imp</em> (6 (12))</th>
<th><em>spn+Sim</em> (1 (2))</th>
<th><em>Imp</em> (0 (0))</th>
<th>Total (47 (94))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jenin (50)</td>
<td>3 (6)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>15 (30)</td>
</tr>
<tr>
<td>Tulkarm (29)</td>
<td>10 (34.5)</td>
<td>11 (37.9)</td>
<td>0 (0)</td>
<td>1 (3.4)</td>
<td>22 (75.9)</td>
</tr>
<tr>
<td>Total (79)</td>
<td>50 (63.3)</td>
<td>17 (21.5)</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
<td>69 (87.4)</td>
</tr>
</tbody>
</table>

Multiple β-lactamase producing isolates have been detected (data not shown).
ERIC-PCR analysis of 35 isolates from Jenin Hospitals which carried genes for MBLs were genetically diverse and comprised a heterogeneous population with a total 16 ERICPCR clusters at a (50%) similarity level. Data are presented in Figures 2 and 3.

**Figure 1.** A. Multiplex PCR profile specific for MBLs. L: Lanes ladder; Lanes 1-4 for Spm gene (271 bp), lane 5 for Spm gene (271 bp) and Sim gene (570 bp), lane 6,7 and 9 for Imp gene (188 bp) and Spm gene (271 bp) and lane 9 for negative control. B. It is the same as A but bands are demarcated to be obvious.

**Figure 2.** DNA fingerprints generated by ERIC-PCR analysis of 35 clinical E. coli isolates carried genes for MBLs recovered on 1.5% garose gel. L: Lanes contained ladder, while other lanes for ERIC-PCR products.

**Discussion**

The increase in the rates of antibiotic resistance is a major cause for concern in isolates of the *Enterobacteriaceae* family. Beta-lactams antimicrobial agents are considered one of the main treatment for serious infections. Carbapenems are considered the most active of these antibacterials, which can be used for the treatment of infections caused by ESBL-producing microorganisms, particularly *Escherichia coli* [7]. The early detection of β-lactamase producing microorganisms would be important to avoid the intrahospital dissemination of such strains and to reduce death rates among patients [16].

Studies on carbapenemase producing *E. coli* were very limited when compared to those on non-fermenters. In Palestine there are no studies about MBL producing microorganisms till now. Results of this study showed that the prevalence of MBLs among *E. coli* in North of Palestine using multiplex PCR technique was 87.4%. In other countries, the prevalence of MBL producers *E. coli* ranged from 1.7%-45.2% [12,14,18,24,26]. Finding of this re-
The high prevalence of MBLs producers among E. coli isolates in Palestine may be due to several risk factors such as long term exposure to antibiotics in hospitals, prolonged hospitalization, incorrect therapy, nursing home residency, severe illness, catheterization and movement of health staff in the hospital leading to dissemination of these pathogens throughout the hospital [38,39]. Results of the study showed a differences between the prevalence of MBLs between the 2 governorates, Tulkarm and Genin, which are both located in North of Palestine. Geographical variation in the occurrence rate of β-lactamases production have been detected from different countries and even from hospital-to-hospital within the same country [40-42]. Multiple β-lactamase has been detected in many of these isolates (data not shown) and a total of 26.1% of MBL producers carried 2 genes, this was an alarming finding.

The coexistence of different classes of β-lactamases in a single bacterial isolate may pose therapeutic challenges, this will seriously limited treatment options. In addition, may pose diagnostic challenge that high-level expression of certain β-lactamases such as the AmpC β-lactamases may mask the recognition of the ESβLs and it may result in a fatal and an inappropriate antimicrobial therapy [43]. The presence of AmpC β-lactamases and ESβLs in a single isolate decreases the effectiveness of the β-lactam-β-lactamase inhibitor combinations, while MBLs and AmpC β-lactamases confer resistance to carbapenems [44]. In addition, coexistence of different classes of β-lactamases in a single bacterial isolate poses a serious concern for infection control management, associated with increases in length and cost of hospital stays [23]. Coexistence more than one type of β-lactamases was reported from different species of Enterobacteriaceae including E. coli [28,30,44,45,46].

The ERIC-PCR typing of MBLs-producing isolates showed various DNA banding profiles. This clonal diversity suggests that most of the strains have been unable to be maintained or spread in different settings of hospital. This observation challenges many conventional thoughts about the nosocomial epidemiology of antibiotic resistance including β-lactamase. These isolates recovered mostly from urine of patients treated mainly in hospitals, sharing significant patient demographics (all isolates used in ERIC-PCR typing are from patients from Jenin Governorate) and isolate characteristics including antibiotic resistance profiles differed. It is clearly indicates that multiple clones of these β-lactamases producing isolates were widespread in these hospitals but not sporadic. This supporting the suggestion that the high rate and extensive inappropriate use of antibiotic especially cephalosporins in the country could be the only major cause [46].

MBLs producing Gram-negative bacteria are an increasing public health problem worldwide because of their resistance to all β-lactams except Aztreonam. In conclusion, our results showed high occurrence of MBLs among E. coli isolates in Palestine. Based on these results we recommend the continuous monitoring and surveillance of the prevalence, proper control and prevention practices and effective antibiotic use will limit the further spread of MBLs producing isolates within hospitals in this country.

Figure 3. Dendrogram of 35 E. coli isolates carried genes for MBLs based on the UPGMA method derived from analysis of the ERIC-PCR profiles at a 50% similarity level. C: Cluster

The search showed that Spm-type MBL was the most common in E. coli isolates and this result is inconsistent with other studies in Japan [30], which showed that 100% of MBL producing E. coli carried IMP gene. The most widespread MBLs in other bacterial species include IMP, VIM, and NDM [5].
Conflict of Interest
We declare that we have no conflict of interest.

References


